

Examples of bacteria that can be used for positive control:

1. *Klebsiella pneumoniae*
2. *Pseudomonas aeruginosa*
3. *Escherichia coli*

Preparing Bacterial Standards

- I. Culture a Gram negative bacteria sample so it is 16-18 hours old (not more than that) when it is ready to use. Use 5% Sheep's blood agar as culture medium.
- II. Suspend a loop of bacteria into sterile water to match a CFU standard. (For example, using a spectrophotometer $A_{600} = 0.12$ is equivalent to a 0.5 McFarland Standard, or 1.5×10^8 CFU for *E.coli*.)
- III. Perform a 10-fold serial dilution keeping the final volume at 1 mL. (For example, taking 100 μ L of a 10^4 CFU sample into 900 μ L of sterile water gives you a 1000 CFU sample). To test for specific low CFU counts, like 1 CFU for example, you would need to make a specific dilution at 1 CFU/500 μ L. To keep the final volume of the dilution at 1 mL, this would mean making a 2 CFU/1mL dilution. To follow this example, you could take 200 μ L of a 10 CFU/mL sample and add 800 μ L of sterile water to it to get 2 CFU/mL.
- IV. Verify CFU level by plating a full mL of the dilution level, and incubating for 24-48 hours at 37°C. Count the colonies formed.

Positive Control Testing

- 1) Don proper PPE as a measure of good practice.
- 2) Pipette 0.5 mL of the above final dilution using a pipette.
- 3) Add the 0.5 mL of water to the provided sterile cuvette with the growth medium. Close the lid. Invert gently a couple times for mixing.
- 4) Place vials in the block incubator and allow 12 or more hours of incubation. The incubator should be set to 37°C.

- 5) After incubation, the cuvette needs to be allowed to cool down. One of two methods can be employed:
- 6) Cooling Sample
 - a. Room temperature cooling: remove the cuvette and place in the supplied holder and allow cooling for a minimum of **1 hour**, but not greater than 3 hours. Continue to the next step.
 - b. Refrigerator cooling: remove the cuvette and place in the supplied holder. Place in a refrigerator (approximate temperature of 4C) for **15 minutes**. Remove from the refrigerator at 15 minutes and continue to the next step.
- 7) Before adding Reagent A, switch the power source of the fluorometer at the upper right corner to "ON".
- 8) Add 2 drops of Reagent A to the cuvette.
- 9) *'Gently' invert it about four times to help mix the Reagent A with the sample. While inverting hold the cuvette at its top half. Make sure there are no air bubbles. Immediately proceed to the next steps for testing.*
- 10) Place the cuvette in the fluorometer, line up the pointy side of the cuvette with the black line in the reader, and place the black cap firmly on the fluorometer.
- 11) This screen will show up, press the "Measure" button.
- 12) Press "Ch 360".
- 13) Press "Blank" (timer will start counting seconds).
- 14) Press "Measure" and wait 10 minutes to get the reading.
- 15) At 10 minutes, the fluorometer will automatically take a reading. (A value will be displayed in the box below the timer). The value displayed before 10 minutes is disregarded. Please Note: The timer on the fluorometer will continue to run, but the reading displayed is taken exactly at the 10 minute mark.
- 16) If desired to test a new sample, press "Return" twice to begin a new sample.

INTERPRETATION OF RESULTS

- A numerical value greater than 300 indicates the presence of gram negative bacteria.
- If a value of less than 300 is reported, conduct another positive control test.
- If the second test is also below 300, contact Healthmark.